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Transport by the Lactose Permease of *Escherichia coli* as the Basis of Lactose Killing

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Lactose killing is a peculiar phenomenon in which 80 to 98% of the *Escherichia coli* cells taken from a lactose-limited chemostat die when plated on standard lactose minimal media. This unique form of suicide is caused by the action of the lactose permease. Since uptake of either lactose or galactose by the lactose permease causes death, the action of rapid transport across the membrane must be the cause of the phenomenon. Alternative causes of lactose killing, such as accumulation of toxic metabolic intermediates or action of the β-galactosidase, have been eliminated. It is proposed that rapid uptake of sugars by the lactose permease disrupts membrane function, perhaps causing collapse of the membrane potential.

Environmental resources in nature commonly vary between shortage and excess. Thus, one expects organisms to have become well adapted to fluctuations in their nutrient supply. In most cases, organisms are indeed adapted to short-term fluctuations in nutrient supply. With respect to fluctuations in the amount of lactose, however, *Escherichia coli* cells are anything but adapted. Indeed, when *E. coli* cells that have been growing on a limited supply of lactose in a chemostat are provided with excess lactose, they stop growing (13). If these same cells are provided with excess glucose or maltose, they continue to grow. Trivial explanations of the phenomenon, which has been called lactose killing, such as those based on changes in media pH or ionic strength, have been eliminated (24). We therefore decided to examine the physiological basis of the lactose effect.

Substrate-accelerated death (3, 4, 18, 19, 22, 23) is a phenomenon similar to lactose killing. It is induced experimentally by growing *Aerobacter aerogenes* in energy-limited chemostats, washing the cells, and then starving them in buffer either with or without the energy source. (The cells are unable to grow because the buffer lacks magnesium, nitrogen, and other factors.) Under these conditions, cells die faster in the presence of the energy source to which they were adapted than they do in its absence, with glycerol and lactose being particularly effective in accelerating death.

For both lactose killing and lactose-accelerated death, the cells were grown in chemostats where lactose is limiting growth. The cells physiologically best adapted to these chemostats must be those cells which have turned off any regulatory system that modulates the entry and use of lactose. When these cells are given excess lactose, an excessive and unregulated uptake and metabolism of lactose must result, leading to the lethal effects. We are interested in finding the cause of the lethality.

Unregulated glycerol metabolism is also lethal in *E. coli* (8). When both repression and feedback inhibition of glycerol kinase are eliminated by mutation, metabolism of glycerol is toxic. Methyl-glyoxal, produced from glycerol in these unregulated cells, causes this toxicity. Another toxic cellular intermediate that could cause lactose killing is UDP-galactose. Experiments show that accumulation of neither of these metabolic intermediates could account for lactose killing. After considerable data were collected on the phenomenology of lactose killing, it was concluded that the entry of lactose via the *lacY* permease is the cause of lactose killing. This hypothesis was confirmed by a final experiment. We showed that a strain lacking β-galactosidase but with a constitutively expressed lactose permease is also killed by lactose.

MATERIALS AND METHODS

Standard techniques for the handling and genetic manipulation of *E. coli* were used (e.g., 16). Media and chemostat apparatus have been described previously (7).

Strains of *E. coli* K-12 used were: (i) DD323 *rpsL* (Dykhuizen and Davies, unpublished data). (ii) C249 *rpsL his* Δ(*bio-gal*) (6). (iii) DD539 HfrC *lacI*$^{-}$*Z*$^{\Delta W4680}$*Y*$^{+}$ *ebgRA*$^{+}$, a derivative of A2 of Hall and Hartl (10) selected for growth on lactose without added isopropyl-1-thio-β-galactoside (IPTG). *ebgR* codes for the repressor (10). (iv) DD552 *rpsL his*, a *gal*$^{+}$ *bio*$^{+}$ derivative of C249 obtained by P1 transduc-

tion. (v) Lin 1 *phoA8 relA1 tonA22* T2^r obtained from B. Bachmann (8). (vi) Lin 292 *phoA8 relA1 tonA22* T2^r *mgr2*, a methyl-glyoxal-resistant derivative of Lin 1, obtained from B. Bachmann (8). (vii) DD654 *lacZ*$^{\Delta W4680}$ *rpsL*. (viii) DD655 *lac*$^+$ *rpsL*, a *lac*$^+$ derivative of DD654 obtained by P1 transduction. (ix) CSH66 Δ*lac thi* (λcI857S7p*lac5IZ*$^+$Y) (14). (x) W3102 *galK2*, obtained from B. Bachmann.

Enzyme assays for β-galactosidase were performed according to Pardee et al. (17); assays for galactokinase were performed according to Heinrich and Howard (11). Assays for β-galactoside permease were the same as those for β-galactosidase except that intact rather than toluenized cells were used, since the amount of β-galactoside permease limits the rate of substrate hydrolysis in intact cells (21).

Assays for the amount of lactose killing were performed by sampling from the chemostat overflow on days 6, 7, and 8 after the chemostats were inoculated. These samples were diluted appropriately and plated on six plates each of maltose, melibiose, galactose, and lactose minimal medium supplemented with required nutrients. Since microscopic cell counts showed that virtually all (95 to 99%) of the cells survived on maltose, the amount of lactose killing was measured as the reduction in survival when plated on other sugars as compared to survival on maltose. Sampling the chemostats on days 6, 7, and 8 represents our empirically determined optimum procedure. Lactose constitutive mutants are rapidly selected in lactose-limited chemostats, reaching very high frequencies after day 3 or 4 (Dykhuizen and Davies, unpublished data). One wishes to sample after this radical change has taken place but before other unrelated evolutionary changes have occurred in the chemostats.

In some experiments, such as the one to determine whether cells can be revived after incubation in lactose, a series of 10-mm-diameter test tubes were used. Samples were diluted 10^9-fold into the growth media, and 0.5-ml samples were dispensed into the tubes. With cell densities as they are in our chemostats, this procedure leads to less than a 50% chance that a given tube will contain one or more cells. After 2 days of incubation of the control (broth-containing) tubes at 37°C, the proportion of tubes with no growth was assumed to equal the null term of a Poisson distribution; from this, the number of viable cells per milliliter in the original sample was estimated, and the percentage of killing was thereby calculated from proportion of experimental tubes in which no cell growth occurred.

A different procedure was devised to assay the lactose killing in the *lacZ* deletion strain (DD654) because the survivors will not grow on lactose minimal medium. The diluted cells plus 2.5 ml of soft agar (0.2% lactose + 0.5% agar in Davis salts) was poured onto lactose minimal medium plates (0.2% lactose + 1.4% agar in Davis salts). These plates were incubated for 2 days and then overlaid with 5 ml of soft agar containing 1% maltose. After 2 days, the visible colonies were counted as the surviving cells. No more colonies appeared with 6 days of additional incubation.

To assay for lactose constitutivity, cells were transferred from colonies growing on the maltose minimal medium plates onto TTY plates (12) with sterile toothpicks. The TTY plates were incubated overnight. The next morning a drop of toluene was put on each colony. After 15 min, the toluene was evaporated with forced air, and a drop of *o*-nitrophenyl-β-D-galactopyranoside (0.4 mg/ml) was put on each colony. The constitutive colonies turned dark yellow; the regulated ones remained white. It was not possible to assay strain DD654 directly for constitutivity, since it contained no β-galactosidase. The following genetic manipulation was done so this strain could be assayed. A λp*lac* lysate was made from CSH66. These phage were cross-streaked on MacConkey-lactose plates (16) against cultures grown from single cells of DD654 taken from the chemostat. Cells that were phenotypically Lac$^+$ were picked from the intersection of the streaks. If a cell carrying the phage is still repressible, the repressor from the cell will repress the β-galactosidase gene carried by the phage. If the cell is constitutive, the β-galactosidase carried by the phage will not be repressed. Thus the assay for constitutivity using *o*-nitrophenyl-β-D-galactopyranoside can also be used on lysogenic cells from strain DD654.

RESULTS

The phenomenology of lactose killing. When *E. coli* were grown for 6 to 8 days in a lactose-limited chemostat (0.02% lactose), 80 to 98% of the cells were killed when plated on lactose minimal medium (0.2% lactose). Strain differences explain much of the variability in the percentage of lactose killing. For example, the killing rate of DD552 was about 85%, whereas the killing rate of W3102 was 98%. These strains are also different in the amount of β-galactosidase in the cells. DD552 cultures contained about 60% of the activity found in W3102. A somewhat larger percentage of the cells survived if casein amino acids were included in the plates, and various culturing and handling procedures had a minor effect on survival rate. *E. coli* cells grown in chemostats limited for glucose, maltose, or galactose exhibited no such killing phenomenon when plated on their respective sugars or on lactose. Indeed, lactose killing was provoked by lactose itself. When *E. coli* cells grown in a lactose-limited chemostat were plated on maltose minimal medium, no killing occurred. Moreover, cells that had grown overnight on maltose plates could be transferred to lactose plates without any mortality. This result suggests that the most important factor in lactose killing is the physiological state attained by cells in the chemostat, although genetic changes might also influence the phenomenon. The importance of the physiological state is confirmed by the following experiment. Cells from a lactose-limited chemostat were grown for 2 h in glucose minimal medium, at which time the cells had undergone slightly more than one division. When these cells were then plated on lactose

minimal medium, lactose killing was no longer observed. Thus, after one generation of growth on glucose, the physiological state of the cells was sufficiently altered to prevent lactose killing. This suggests that the normal balance and interaction of the pathways involved in lactose catabolism somehow become distorted by growth in a lactose-limited chemostat.

In a related experiment, four sets of 72 test tubes each were inoculated with such a dilution of a chemostat culture that the mean number of cells per inoculum was less than 1. One control set of tubes (A) contained 1 ml each of glucose nutrient broth; a second set (B) contained 1 ml each of nutrient broth plus 0.5 ml of 0.2% lactose minimal medium; a third set (C) contained 1 ml each of glucose nutrient broth, to which was added 0.5 ml of 0.2% lactose minimal medium 10 min after inoculation; a fourth set of tubes (D) contained lactose minimal medium and served as another control. The number of tubes in group A producing growth was that expected on the hypothesis of no killing. Relative to these, survival in control set D was 15%. Consequently, the killing is not mediated through medium effects, because single cells in the tubes with 0.5 ml of lactose minimal medium died: sets B and C both had 37% survival relative to control A. Thus, growth in rich medium offers some, but not much, protection against lactose killing. This suggests that a nutritional or energy shortage is not the cause of the killing.

The question whether lactose actually kills the cells can be approached by finding out whether cells exposed to lactose can be revived by the addition of rich medium, since more cells survive exposure to lactose in a rich medium than in minimal medium. Accordingly, a sample from a lactose-limited chemostat was diluted as above into various media and added to a series of small test tubes. Table 1 shows the number of tubes (out of 72) showing growth after 2 days on maltose minimal medium, lactose minimal medium, or a balanced salt solution (Davis salts). As shown in the Table, 37/72 or 52% of the maltose-containing tubes contained viable cells, which corresponds to a mean number of cells per inoculum of 0.73. Only 8 of 72 tubes showed growth in lactose minimal medium, so the mean number of surviving cells per inoculum is 0.12. This gives a rate of lactose killing of 0.61/0.73 or 84%. In salts alone, of course, there was no growth. To determine whether cells exposed to lactose for 2 days could be revived, 1 ml of L broth was added to each tube, and the number of additional cultures that grew were recorded. As shown in Table 1, no additional tubes containing maltose showed growth, whereas virtually all of the cells maintained in salts survived and grew in the presence of broth. As indicated in the third row of Table 1, no additional growth was observed when an identical set of lactose cultures were plated individually on L broth plates containing glucose to dilute out the lactose. We conclude that 2 days in lactose minimal medium causes cell death.

TABLE 1. *Revival of cultures exposed to lactose*[a]

Medium	No. of cultures showing growth	Additional cultures
Maltose	37	0
Lactose	8	0
Lactose	8	0[b]
Salts	0	36

[a] Number of cultures (out of 72) that exhibited growth on the various media shown, and the additional number of cultures that grew after 1 ml of L broth was added at 48 h. Cells maintained for 48 h in lactose minimal medium cannot be revived with L broth.

[b] The remaining 64 tubes were individually spread onto L plates.

There is a strong positive correlation between the amount of β-galactosidase per cell and the rate of lactose-accelerated death in *Klebsiella aerogenes* (4). This is also true for lactose killing (for evidence see Table 2). Since the amount of lactose permease is strongly correlated with the amount of β-galactosidase (they are coordinately controlled), there is also a strong correlation between the amount of lactose permease and the amount of lactose killing.

Wild-type *E. coli* in a lactose-limited chemostat usually becomes constitutive for the *lac* operon after 3 or 4 days, owing to the selective advantage of constitutive mutations (Dykhuizen and Davies, unpublished data). Before the constitutive mutant takes over, the amount of β-galactosidase is low (2.9 units) and there is no killing. After the constitutive mutation takes over, the amount of β-galactosidase is high (7.5 to 12 units) and the rate of killing is high (80 to 98%). If a lactose-limited chemostat was inoculated with a lactose-constitutive strain instead of a *lac*$^+$ strain, high killing rates (70%) were seen 1 day after inoculation. By days 6 to 8, the average killing rate for this strain was 93%.

Growth of *E. coli* in melibiose-limited chemostats led to lactose killing (Table 2). This is because *lac* constitutives are selected in such chemostats because melibiose, an α-galactoside, is transported into the cell by the lactose permease (12). Indeed, the rate of lactose killing parallels the increase of *lac* constitutives in the chemostat, as shown in Fig. 1. That the correlation is causal is indicated by the fact that, on day 7, 84% of the chemostat cells were *lac* constitutives, but only 75% of the survivors on lactose plates were.

TABLE 2. *Amount of killing of various strains on lactose, galactose, and melibiose minimal media as a function of the degree of induction of the lactose and galactose operons*

Strain[a]	Chemostat medium	Units of enzyme		% Killing on[b]		
		β-Galactosidase	Galactokinase	Lactose	Galactose	Melibiose
DD323	Galactose	0	0.12	0	0	1
C249	Lactose	3.4	0	4		0
C249	Glucose + IPTG	4.7	0	37		4
DD323	Galactose + IPTG	5.4	0.11	49	9	3
DD323	Glucose + IPTG	5.7	0.02	52	20	4
DD323	Melibiose	5.9[c]	0.08	60[d]	8	6
DD552	Lactose	7.9	0.08	86	33[d]	8
DD323	Lactose	9.3		82	36[d]	13
DD671	Glucose + IPTG	13.8		98		8
DD539	Lactose	0.02	0.04	43	14	3

[a] DD323 and DD552 are wild type for the *lac* and *gal* operons; DD539 is *lacI*$^{-}$ $Z^{\Delta W4660}Y^{+}$ *ebgA*$^{+}$*R*; C249 carries a deletion of the *gal* operon.

[b] Percentage of killing was calculated as 1 minus the percentage of survival, where percentage of survival is expressed relative to survival on maltose minimal medium. Survival on maltose minimal medium was virtually 100%.

[c] The activity increased from 5.0 units on day 6 to 6.8 on day 8; the number in the table is the average.

[d] The percentage of killing increased from day 6 to day 8; the numbers in the table are averages.

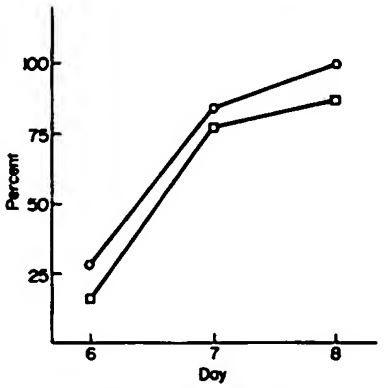

FIG. 1. *Relationship between degree of lactose killing and proportion of constitutive lac cells in a melibiose-limited chemostat.* (□) *Percentage of cells killed when plated on lactose minimal medium;* (○) *percentage of cells in the chemostat that are constitutive for the lactose operon.*

Are the survivors of lactose killing genetically resistant to the phenomenon? This was tested by examining two isolates that had survived a level of 88% lactose killing. Both strains were constitutive for *lac*, and when grown in lactose-limited chemostats produced the same amount of lactose killing as found in controls. Thus, survival seems to be a physiological phenomenon, although we cannot eliminate the possibility that the resistant isolates were genetically resistant at the outset but underwent replacement by sensitive revertants under selection in the chemostat.

Methyl-glyoxal. Since methyl-glyoxal, a small diffusible molecule, has been found in the media of mutant cells (Lin 1) fed glycerol, cells from a lactose-limited chemostat were exposed to lactose for 2 h to see if any toxic compound could be found in the media. We found no evidence of any such compound. We then made a more direct test of the hypothesis that methyl-glyoxal caused lactose killing. Strains Lin 1 and Lin 292, which are different only in that the latter strain is resistant to 50 to 75 nM methyl-glyoxal, were grown in lactose-limited chemostats. The killing rates for Lin 1 were 94% on lactose and 94% on methyl-glyoxal; whereas, the killing rates for Lin 292 were 93% on lactose and 30% on methyl-glyoxal. Thus, while the strains retain their different sensitivities to methyl-glyoxal, the two strains are equally sensitive to lactose. This shows methyl-glyoxal is not the cause of lactose killing.

UDP-galactose. Next we considered the hypothesis that the metabolism of galactose is the cause of lactose killing. A prime suspect for the killing would then be UDP-galactose, a highly toxic intermediate formed in galactose metabolism. Cells defective in *galE*, the gene coding for the enzyme whose substrate is UDP-galactose, die in the presence of galactose due to accumulation of UDP-galactose. However, cells grown in galactose-limited chemostats survive when plated on galactose. If UDP-galactose or any other metabolite of galactose is involved in lactose killing, this must be due to an interaction between the lactose and galactose operons. This interaction can be assessed by examining the correlation between the amount of lactose killing and the degree of induction of the lactose and galactose operons.

Induction of the lactose and galactose operons can be uncoupled by using combinations of isopropyl-β-D-thiogalactopyranoside (IPTG) (a gratuitous inducer of the lactose operon which is effective at concentrations giving only minimal induction of the galactose operon), galactose itself, which induces the galactose operon but not the lactose operon, and glucose, which strongly represses the galactose operon. Table 2 shows the relationship between levels of induction of the lactose and galactose operons and the amount of lactose killing. The amount of killing parallels the induction of the lactose operon but is unrelated to the level of induction of the galactose operon.

A further experiment was done to show that UDP-galactose could not cause lactose killing. Strain W3102, which carries a point mutation of *galK*, the gene which codes for the first enzyme in the galactose catabolism pathway, was tested for lactose killing and found to be very sensitive to lactose, even though no UDP-galactose could be produced.

Galactose. Turning now to galactose itself as the potential basis of lactose killing, it might be argued that the greater the activity of the lactose operon, the greater the intracellular accumulation of galactose. This postulated overload of galactose could cause the sort of correlation shown in Fig. 1.

There are two reasons for excluding galactose as the cause of lactose killing. First, as shown in Table 2, we did not find the negative correlation expected between the activity of galactose kinase and amount of lactose killing. Were intracellular accumulation of galactose responsible for killing, a negative correlation would certainly be expected, as lower levels of induction of the galactose operon would lead to higher accumulated levels of galactose.

A second reason for excluding galactose accumulation as the cause of lactose killing derived from studies of strain DD539. This strain carried a deletion of *lacZ* but it constitutively produces another β-galactosidase from the *ebgA*$^+$ gene. The activity of this β-galactosidase is only about 1% of that of the *lacZ* gene product (10). In strain DD539, which produces the lactose permease constitutively, one expects an accumulation of lactose but not an accumulation of galactose. Hence, in the galactose accumulation hypothesis, little or no lactose killing is expected. The observed amount of killing was, however, 43%. This result also shows that lactose killing is not dependent on the activity of the *lacZ* gene.

Lactose. The accumulation of lactose could not be the cause of lactose killing because β-galactosidase is so much more active than the permease that very little lactose will accumulate within the cell. The amount of lactose killing in strain DD539 also serves to eliminate lactose itself as the cause of lactose killing. Owing to its low level of β-galactosidase activity, this strain should accumulate more lactose than the wild-type strains (DD323) and thus exhibit higher levels of lactose killing than these. The actual level of lactose killing is considerably less than in these wild-type strains.

The uptake of lactose. Taken together, the foregoing results eliminate methyl-glyoxal, UDP-galactose, galactose accumulation, and lactose accumulation as possible causes of lactose killing. That leaves entry of lactose into the cells to be considered, and we now provide evidence that lactose permeation is indeed the cause of the phenomenon. If a strain which has a deleted β-galactosidase shows lactose killing, then no step or product in the catabolism of lactose can be the cause of lactose killing, leaving only entry or uptake as its basis. This idea was tested using strain DD654. In this strain only a portion of the β-galactosidase gene is deleted. The rest of the lactose operon is left intact. Strains DD654 and DD655, the *lac*$^+$ control, were grown in melibiose-limited chemostats and tested on day 8 after inoculation (Table 3). DD654 showed nearly 50% killing. This cannot be because the cells were unable to grow for 2 days. When cells were plated on agar without any sugar, incubated for 2 days, and then grown, there was only 5% killing. The lower percentage of lactose killing in the deletion than the control is explained by the lower percentage of constitutives in the former culture than the latter (see Fig. 1).

Galactose deletion. Strain C249, which carries a deletion of the whole galactose operon, does not exhibit lactose killing when grown in a lactose-limited chemostat. Incidentally, the equilibrium cell densities of C249 are only one-half the densities of wild type on the same concentration of lactose, since C249 is unable to use the galactose half of lactose. The lack of killing in C249 is explainable. The amount of β-galactosidase in these cells is so low that little killing is expected (Table 2). The C249 cells, which had grown in lactose-limited chemostats for 8 days, were checked for lactose constitutive

TABLE 3. *Amount of killing on various sugars in a β-galactosidase deletion strain (DD654) and a lac*$^+$ *control (DD655)*

Strain	% Killing on				% Constitutive
	Lactose	Galactose	Melibiose	No sugar	
DD654	47	44	2	5	52
DD655	87	43	12	14	92

mutations. Out of the 400 tested, no constitutives were found. The lactose operon in strain C249 is normal because strain DD552, which is a *gal*$^+$ *bio*$^+$ transductant of C249, became lactose constitutive and showed normal amounts of lactose killing in lactose-limited chemostats. Also, the galactose deletion is not blocking lactose killing because C249, grown in glucose plus IPTG, showed normal killing (Table 2). Consequently, the presence of the unusable galactose seems to block the selection of constitutive mutations. This could be because the useless galactose in the cell is harmful and a balance must be reached between lactose uptake and galactose disposal.

One chemostat culture of C249 supported the hypothesis that entry of lactose is the cause of lactose killing. This culture was grown in lactose-limited medium, gave no lactose killing (the ratio of the number of cells on lactose plates to the number on maltose plates was 1.03), and showed a level of β-galactosidase activity (3.9 units) for which 15% killing was expected. It was found that the activity of the lactose permease of cells from this chemostat was one-half that of cells from the C249 stock culture or from other chemostats, whereas the activity of β-galactosidase in all these cells was the same when grown in shaker culture with IPTG. Thus a genetic change which lowers the amount of lactose permease protects against lactose killing.

DISCUSSION

The phenomenon of lactose killing seems to be unique. *E. coli* cells grown in chemostats limited for other sugars exhibit no similar sensitivity. However, since not all sugars were tested, glycerol or some other sugar may produce a similar sensitivity.

In this paper we have eliminated the possibility than any catabolic product of lactose causes lactose killing. The thiogalactoside transacetylase, the *lacA* enzyme, cannot be the cause of lactose killing because this transacetylase will not accept lactose as a substrate (1). The β-galactosidase cannot be the cause of lactose killing, because *lacZ* cells still show lactose killing. The presence of lactose in the cell is not the cause of the killing, because cells grown in lactose-limited chemostats die on galactose. We propose that the transport of lactose across the cell membrane is the basis of lactose killing. There is also evidence that the misfunction is at site I of the lactose permease. The lactose permease (the so-called M protein from the *lacY* gene) is a large protein of about 30,000 molecular weight (9). There appear to be two principal sites of uptake, site I, which takes up lactose and galactose, and site II, which takes up the α-galactoside, melibiose (15). If site I were involved in lactose killing but site II were not, then one would expect considerable galactose killing whenever galactose is primarily transported by the lactose permease, but not much melibiose killing. This is what was seen (Tables 2 and 3). The rate of melibiose killing was generally low, on the order of 1/5 to 1/10 the rate of lactose killing. On the other hand, the amount of galactose killing can be substantial, up to 1/2 the rate of lactose killing. There is, however, no galactose killing in the absence of induction of the *lac* operon.

Our interpretation of the mechanism of lactose killing is at variance with that of Horuichi et al. (13). They concluded that the activity of β-galactosidase was a prime factor in the killing because IPTG, a non-metabolizable β-galactoside, which is transported by site I of the lactose permease, did not stop growth. They grew their hyper-induced cells on sodium lactate in shaker culture and found that addition of lactose stopped growth, whereas addition of the same amount of IPTG only slowed the growth rate by 20%. But von Hofsten (24), who found that cultures of lactose constitutive mutants growing on lactate were not sensitive to lactose, found that addition of lactose stopped growth of lactose-constitutive cells growing on succinate while addition of galactose only slowed down the growth rate of the culture. The percentage of decrease of the growth rate increased with increasing amounts of galactose. Thus, while the result of Horuichi et al. (13) is difficult to interpret in terms of the results of the experiments of this paper, it seems that IPTG will behave like galactose in that it will give amounts of killing less than lactose but still significant.

Although our results show that lactose-sensitive cells maintained in lactose for 2 days cannot be revived, death is by no means instantaneous. von Hofsten (24) and Horuichi et al. (13) have shown that such cells can be revived for up to 6 h and that during this time a considerable amount of UV-absorbing material is excreted from the cells. Indeed, a number of factors can alter the amount or timing of lactose-induced death. Calcott et al. (2) have found that magnesium ions (1 mM) or theophyllin (0.5 mM), which prevents breakdown of cyclic AMP, greatly decrease the rate of lactose killing associated with substrate-accelerated death in *A. aerogenes*. Cells starved in lactose medium seem to extrude cyclic AMP into the medium even though the internal concentration of cyclic AMP is low and the cells are producing cyclic AMP. However, this also happens when glucose is added to starved cells grown on glucose (20) and there is no similar killing with glucose. High

external concentrations of cyclic AMP (5 mM) can retard lactose-accelerated death (2). The mechanism of the effect of cyclic AMP is not known, but it may be due to a regulatory effect on the lactose permease.

Until lactose killing is better understood, any explanation of the physiological mechanism will be speculative. However, we will propose a possible mechanism, which is that the rapid uptake of sugars causes collapse of the membrane potential. Lactose uptake is driven by a proton gradient, and passive accumulation of weak acids reflects the presence of this gradient (14). Kaback (14) reports that when lactose is added to membrane vesicles 50% of the accumulated acetate is released even though there is a 60% inhibition of the lactose permease in this system. This implies that the uptake of lactose can rapidly deplete the proton gradient. Consequently, rapid accumulation of lactose in fully induced cells from the chemostat that are physiologically adapted to low sugar levels could collapse the proton gradient and prevent any reestablishment of the gradient.

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